Low-dose responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin in single living human cells measured by synchrotron infrared spectromicroscopy

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INTRODUCTION

Exposure to polychlorinated aromatic compounds can lead to various health effects including cancers, alteration of hormone levels, and reproductive defects in animals[1-6] and humans.[7-14] Among this family of pollutants, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is one of the most potent and most studied "man-made" toxins, causing harmful effects at exposure levels hundreds or thousands of times lower than most chemicals of environmental concern.[15] TCDD acts by binding to the aryl hydrocarbon (Ah) receptor.[16, 17] Binding triggers induction of various genes involved in xenobiotic metabolism including the cytochrome P4501A1 (*CYP1A1*) gene.[16-20]

Synchrotron radiation-based FTIR spectromicroscopy provides several hundred times higher brightness than conventional IR sources at a diffraction-limited spatial resolution of 10 μm or better, and is therefore a sensitive analytical technique capable of providing molecular information on biological specimens.[21-25] In this study we use SR-FTIR spectromicroscopy to measure directly intracellular responses to TCDD.

EXPERIMENTAL DETAILS

The experiments began with exposing HepG2 cells (derived from a human hepatocellular carcinoma) to TCDD at environmentally relevant concentrations. A fraction of the exposed cells were investigated by acquiring SR-FTIR spectra from individual live cells. The remaining cells were analyzed for *CYP1A1* gene expression, using the reverse transcriptase polymerase chain reaction (RT-PCR) technique. More details can be found in Holman, et al..[26]
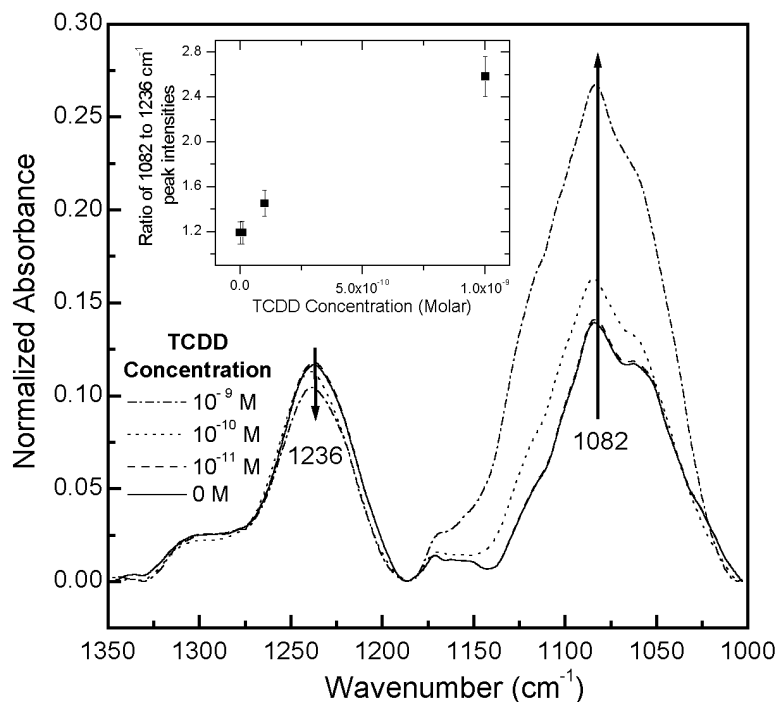

RESULTS

The overall absorbance spectral features of biological materials are well known[27-30] and our cellular SR-FTIR spectra follow the established pattern. However, by comparing spectra from cells treated with various amounts of TCDD to untreated cells we find significant spectral differences in the magnitude and in some cases the location of peaks at various wavelengths.

Phosphate Bands. Figure 1 shows the IR spectra of unexposed HepG2 cells (solid line) and of cells exposed to different concentrations of TCDD

in the phosphate band region. For untreated cells the two phosphate absorption bands[29, 31] at 1236 cm^{-1} (asymmetric phosphate stretching mode ν_{as} PO$_2^-$) and at 1082 cm^{-1} (symmetric phosphate mode ν_s PO$_2^-$) are approximately equal in strength. For TCDD-treated HepG2 cells, the ν_{as} PO$_2^-$ band decreases in intensity while the ν_s PO$_2^-$ band increases by more than a factor of two at the highest TCDD doses studied. The inset to Figure 1 shows the intensity ratio of the ν_s PO$_2^-$ to ν_{as} PO$_2^-$ peaks increases with TCDD concentration. The 1145 to 1190 cm^{-1} region shows a peak that is associated with a C−O vibration[29] that increases in intensity with TCDD concentration.
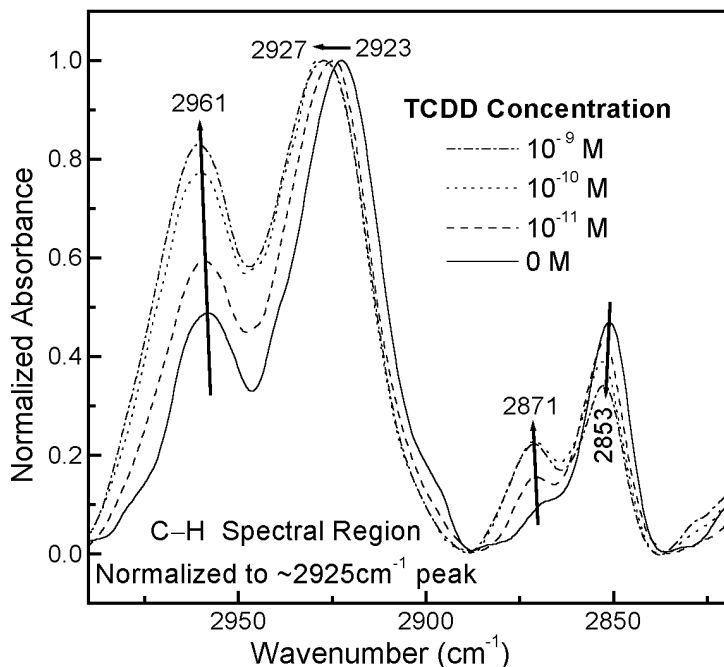
C−H Bands. Spectral absorptions due to hydrocarbon vibrations in lipids, proteins, nucleic acids, sugars, phosphates, among others are found within the 3050-2800 cm^{-1} region. Figure 2 displays the SR-FTIR spectra of unexposed HepG2 cells (solid line) and of cells exposed to different concentrations of TCDD in the C−H stretch region normalized to the peak maximum near 2925cm^{-1}. The band near 2853 cm^{-1} is due to the symmetric CH$_2$ stretching of the methylene chains in membrane lipids; the peak at 2925 cm^{-1} is due to the asymmetric CH$_2$ stretch; 2961 cm^{-1} absorption is due to asymmetric stretching of the CH$_3$ methyl groups of both lipids and proteins; and the 2871 cm^{-1} mode is from the symmetric CH$_3$ stretching mode.[30-32] For TCDD-treated HepG2 cells, the 2853 peak decreases in intensity while the 2961 and 2871 cm^{-1} peaks increase. This indicates that the ratio of the number of methyl groups to that of methylene groups increases as the TCDD concentration increases. The opposite has been found in colorectal cancer tissue analysis.[31, 33] Other authors have proposed that TCDD removes the protection from methylation from certain sites when it binds to the Ah receptor,[34] or increased methylation may downregulate the expression of the *CYP1A1* gene.[35] Since methylation is so intimately involved with gene inactivation,[36] and we observe a significant increase in the number of methyl groups produced after exposure to TCDD potentially indicating increased methylation, this could explain the tremendous toxicity of TCDD in humans and other animals.
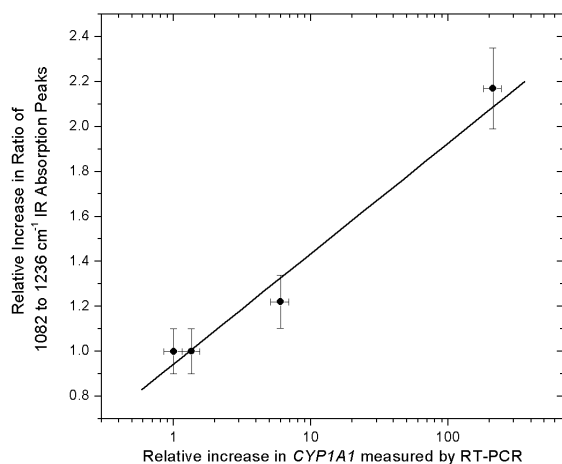
Comparison of SR-FTIR and RT-PCR. RT-PCR was carried out on extracts from the cell cultures of each TCDD exposure. Measured values of *CYP1A1* gene expression were normalized to measured β-actin levels, and finally the relative increase in *CYP1A1* as a function of TCDD was obtained. The relative increase in the ratio of the symmetric to asymmetric phosphate infrared bands with increasing TCDD concentration is compared to the relative increase in *CYP1A1* induction in Figure 3. Error bars for the IR data arise from the fact that we

measured at 5 or less cells for each treatment concentration. The solid line in Figure 3 is a weighted linear regression fit to the data. The excellent agreement (with $r^2 = 0.96$) between the two methods indicates that the rapid SR-FTIR spectromicroscopy technique can measure biochemical changes due to the *CYP1A1* expression processes.

Our data demonstrate the ability of this technique to measure clear spectral changes in cells that have been exposed to environmentally relevant concentrations of TCDD and are expressing the AhR pathway. While the infrared spectra of whole cells are quite complex, and it is extremely difficult to assign the changes observed to specific molecular events, the use of cell lines which are defective in a single process or pathway may allow specific mechanisms to be identified in the spectra and studied comprehensively. Once a better understanding of how to interpret IR spectral changes is accomplished, infrared spectromicroscopy may develop into a rapid and inexpensive diagnostic tool for medical screening applications.

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